

39. (Amended) Mutated *m_{sp}A* gene, wherein the mutated gene is the *synm_{sp}A* gene according to sequence 4.

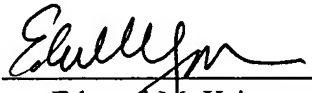
REMARKS

Claim 33 has been canceled herein. Claims 1-5, 7-26, 32, 34-35 and 37-39 have been amended herein. No new claims have been added herein. Therefore, claims 1-32 and 34-41 are under active consideration.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Kriegsman & Kriegsman

By: 
 Edward M. Kriegsman
 Reg. No. 33,529
 665 Franklin Street
 Framingham, MA 01702
 (508) 879-3500

Dated: February 28, 2002

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on _____.

 Edward M. Kriegsman
 Reg. No. 33,529
 Dated: _____

MARKED-UP AMENDED CLAIMS 1-5, 7-26, 32, 34-35 and 37-39

1. (Amended) A Method for producing a channel-forming protein, found in gram-positive bacteria, wherein the channel-forming protein is obtained by
 - a) heterologous overexpression or
 - b) purification from mycobacteria, [whereby] wherein the extraction temperature is higher than 50°C.
2. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein the gram-positive bacterium is one that contains at least one mycolic acid.
3. (Amended) A Method according to [one of the aforementioned claims] claim 2, wherein the bacterium is a mycobacterium, preferably *Mycobacterium smegmatis*.
4. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein the channel forming protein is a porin.
5. (Amended) A Method according to [one of the aforementioned claims] claim 4, wherein the porin is essentially chemically stable against organic solvents.
7. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein the porin is the porin MspA, MspC, MspD, a fragment of one of these porins, a homologous protein from one of these porins or their fragments, or a protein taken from a sequence of one of these porins.
8. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein the heterologous overexpression is realized in *E. coli* or mycobacteria.

9. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein a gene encoding a channel-forming protein, preferably a porin, is [used for the overexpression] overexpressed.

10. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein an *mspA* gene according to sequence 1, an *mspC* gene according to sequence 6, or an *mspD* gene according to sequence 8 is [used for overexpression] overexpressed.

11. (Amended) A Method according to [one of the aforementioned claims] claim 10, wherein a mutant gene derived from the sequences 1, 6, or 8 is [used for overexpression] overexpressed, in which the mutation is essentially so that the chemical and thermal stability, as well as the channel-like structure, correspond essentially with that of MspA, MspC or MspD.

12. (Amended) A Method according to [one of the aforementioned claims] claim 11, wherein the mutation is essentially so that the codon usage of the *mspA*, *mspC* or *mspD* gene is adapted to that of highly expressed genes in *E. coli*.

13. (Amended) A Method according to [one of the aforementioned claims] claim 11, wherein a mutated *mspA*-, *mspC*- or *mspD* gene is used for overexpression where the mutation is essentially so that the G+C content is reduced to less than 66%.

14. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein the *synmspA* gene according to sequence 4[,] is [used for overexpression] overexpressed.

15. (Amended) A Method according to [one of the aforementioned claims] claim 14, wherein a suitable vector [for overexpression in *E. coli*], containing the *synmspA* gene according to sequence 4, is used for overexpression in *E. coli*.

16. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein the channel-forming proteins are produced from the cell wall from gram-positive bacteria using non-ionic or zwitterionic detergents.

17. (Amended) A Method according to [one of the aforementioned claims] claim 16, wherein the detergents used come from the following list: isotridecylpoly(ethyleneglycolether)_n, alkylglucosides, especially octylglucoside, alkylmaltoside, especially dodecylmaltoside, alkylthioglucosides, especially octylthioglucoside, octyl-polyethylenoxide and lauryldimethylaminoxide.

18. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein the extraction temperature is between 80 and 110°C, preferably between 90 and 100°C.

19. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein the extraction time is 5-120 min, preferably 25-35 min.

20. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein a buffer with an ionic strength above 50 mM NaCl or Na-phosphate is used.

21. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein the channel-forming protein is purified by precipitation, particularly using acetone.

22. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein the channel-forming protein is purified using ion-exchange chromatography, particularly an anion-exchange chromatography.

23. (Amended) A Method according to [one of the aforementioned claims] claim 1, wherein the channel-forming protein is purified using size-exclusion chromatography.

24. (Amended) A Method according to [one of the aforementioned claims] claim 1, [wherein one of the aforementioned claims,] wherein the channel-forming protein, produced through heterologous overexpression by raising [the] its local concentration, is renatured.

25. (Amended) A Method according to claim 24, wherein raising of the local protein concentration is realized by electrophoretic enrichment, especially by means of a DC current, by precipitation or adsorption at a suitable surface, especially at a membrane.

26. (Amended) Channel-forming protein from a gram-positive bacterium, produced according to a method according to [one of the aforementioned claims] claim 1.

32. (Amended) Gene, [encoding a channel forming protein according to one of the claims 26-31] wherein the gene is the *mspA* gene according to sequence 1.

34. (Amended) Gene [according to claim 32], wherein the gene is the *mspC* gene according to sequence 6.

35. (Amended) Gene [according to claim 32], wherein the gene is the *mspD* gene according to sequence 8.

37. (Amended) Mutated *mspA* gene, *mspC* gene or *mspD* gene, [in particular] according to claim 36, in which the mutation essentially consists of reducing the G+C content to less than 66%.

38. (Amended) Mutated *mspA* gene, *mspC* gene or *mspD* gene, [in particular] according to claim 36 [or 37], derived from one of the sequences 1, 6, or 8, in which the mutation is such that the chemical and thermal stability, as well as the channel-like structure of the protein is for all practical purposes that of MspA, MspC or MspD.

39. (Amended) Mutated *mspA* gene [according to claim 36 through 38], wherein the mutated gene is the *synmspA* gene according to sequence 4.